THE OCCURRENCE AND NATURE OF ARYLSULFATASES IN PARAKERATOSES*

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The previous work on the occurrence of the arylsulfatases in skin (1) was done before the complex nature of the arylsulfatases was appreciated (10). In addition, there is no information on the occurrence of sulfatases in various parakeratotic lesions.

Because there appears to be an increase of activity in other hydrolytic enzymes in disease processes (1) this following work was undertaken to attempt to determine the occurrence and nature of the arylsulfatases in normal and in diseased epidermis.

MATERIALS AND METHODS

Collection of Tissues

Fresh rat epidermis was obtained from Sprague Dawley albino strain by the stretch method (3) and treated as described below.

Normal human epidermis was obtained by stretch method from surgical specimens from abdominal skin. Specimens from callus were obtained by an electrical scraper.

Scales were collected by scraping the involved areas of skin with a scalpel. The scales so obtained were either utilized immediately or stored in the deep freeze (−15° C) prior to use.

Preparation of acetone powder

The scales or tissue were homogenized in acetone at −15° C in an Ultrahomogenizer (Bühler) for 10 minutes. The homogenate was filtered, extracted for a further 15 minutes with acetone at −15° C filtered and the powder dried and stored at −15° C. This showed no loss of activity over 2 weeks.

Preparation of butanol powder (4)

The scales or tissue were homogenized in *n*-butanol at −20° C (50 ml/gm) and then extracted for 20 minutes and filtered. The powder was then extracted once more with butanol at −20° C and then twice with acetone each for 10 minute periods. The powder was dried and stored as before.

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Rat epidermal preparation

The epidermis obtained was immediately homogenized in 0.5 M acetate buffer at pH 4.9 at 0 for 10 minutes and the whole homogenate utilized.

Calibration Curve (Graph 1)

This was obtained by adding 1 ml solution containing a known quantity of nitrocatechol sulfate to phosphotungstic acid (3.0 ml) and pipetting 2.0 ml of this into 5.0 ml 2.5 N NaOH and reading optical density at 525 mμ in a Zeiss spectrophotometer PNQ II.

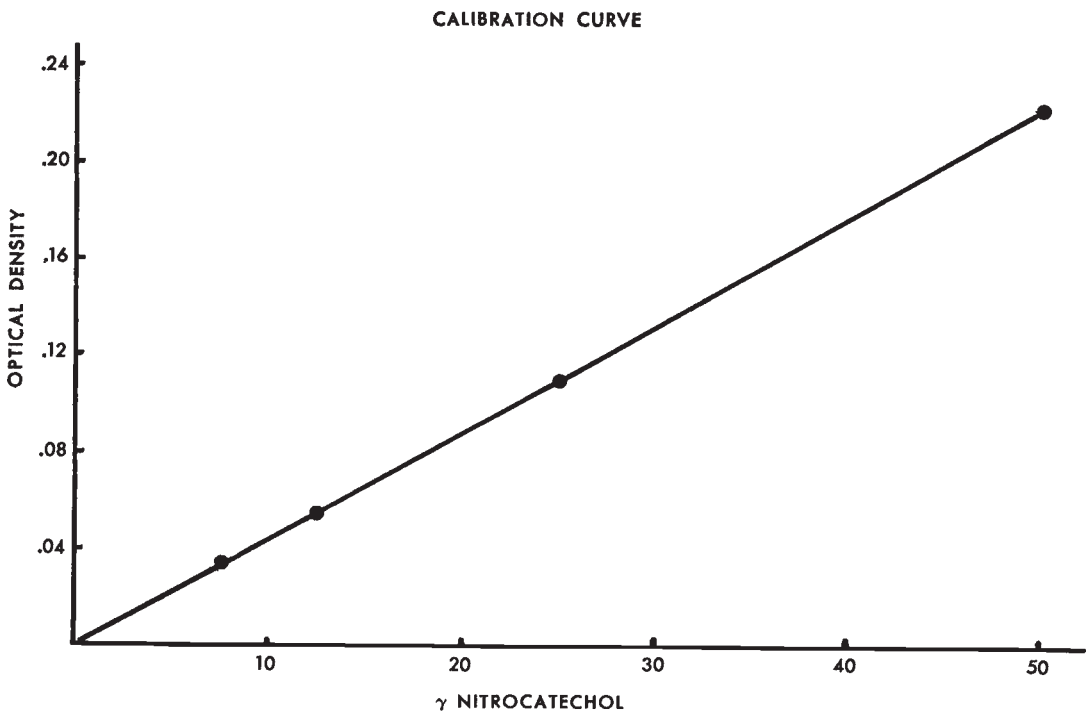
Assay Procedure

Routine assays were carried out by weighing out between 10–15 mgm of the acetone powder into a small homogenizer tube fitted with a loose fitting ground glass pestle. The powder was suspended in 1.0 ml of a solution of 0.006 M nitrocatechol sulfate in 5 M acetate buffer adjusted to pH 4.9. This was incubated at 37° C for 60 minutes with frequent resuspension and 3 ml of 2.5% phosphotungstic acid in 0.1 N HCl added. The protein was centrifuged down and 2.0 ml of the supernatant was pipetted into 5.0 ml 2.5 N NaOH. The color developed was read in the Zeiss spectrophotometer at 525 mμ. Controls were run with immediate phosphotungstic acid addition.

Butanol powder samples were assayed in the same way. Rat epidermis was assayed by incubating 0.4 ml homogenate with 0.6 ml 0.01 M nitrocatechol sulfate in 0.5 M acetate buffer at pH 4.9; color development and estimation being as above. A similar method was used for human epidermis.

Solubilization of Enzyme

The enzyme could not be obtained in solution by buffer or distilled water extraction of either scales or acetone powder. However butanol pretreatment or incubation of the acetone powder with crude bacterial lipase for 2 hrs followed by extraction with distilled water or buffer for 12 hrs at 0° C/and centrifugation at 35,000 g for 25 minutes resulted in a clear supernatant showing enzyme activity. The supernatant was dialyzed against 6 changes of distilled water for 24 hrs at 0° C/and the turbid solution centrifuged and the supernatant stored at −15° C.



GRAPH 1. Calibration curve: 1 ml nitrocatechol into 3 ml phosphotungstic acid and 2 ml of this pipetted into 5 ml 2.5 N NaOH and color read.

0.4 ml of this solution was assayed with 0.6 ml 0.01 M nitrocatechol sulfate in 0.5 M acetate buffer at pH 4.9 or at given pH.

Nitrogen Estimations

These were kindly performed by Mrs. Hollstein using the micro Kjehldahl method.

Synthesis of Substrate

This was carried out according to Roy (5).

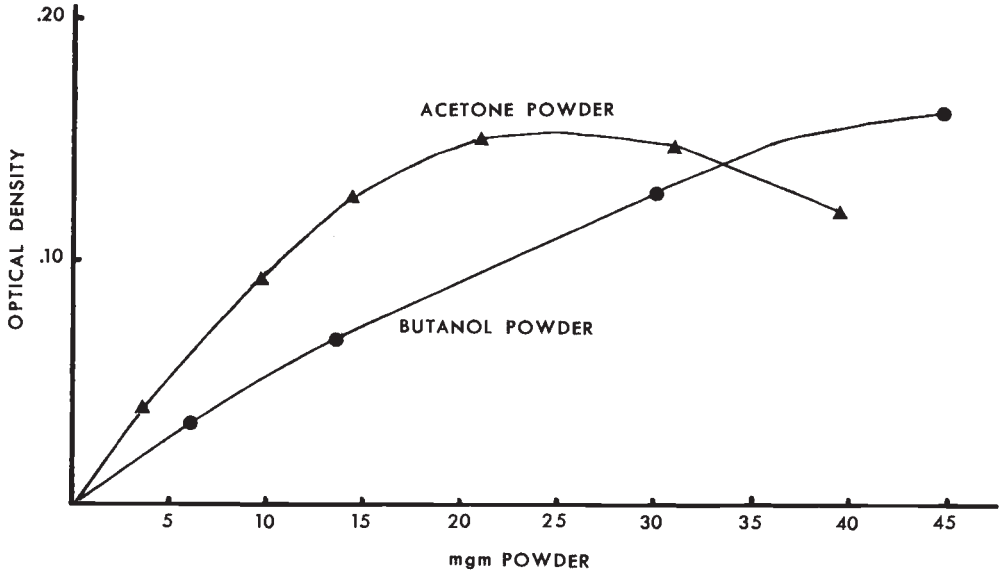
RESULTS

1. Assay Procedure Results

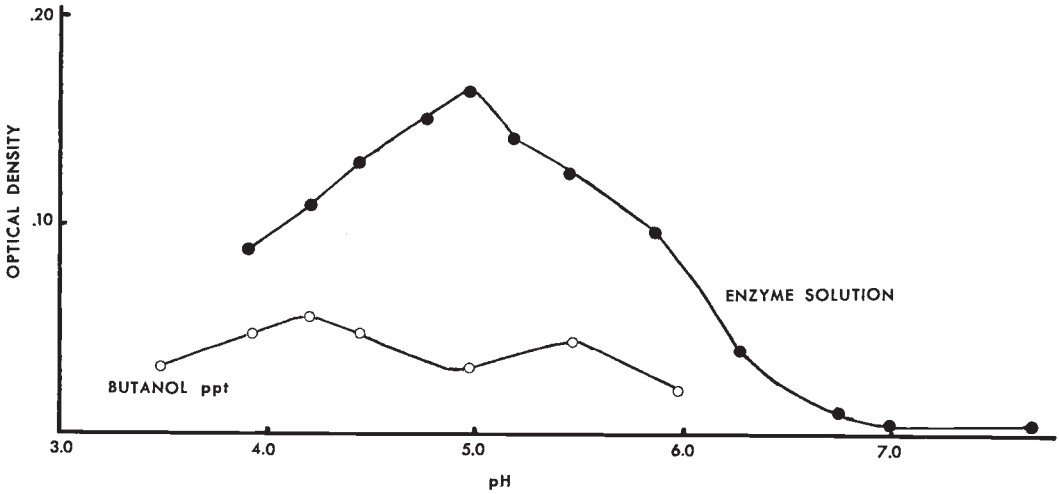
The assay of a preparation of crude sulfatase is susceptible to several errors. The probable mixture of sulfatases presents results in enzymes of varying activity towards a given substrate concentration at a given pH, each of the known sulfatases having a different substrate and pH optimum. Also the nitrocatechol produced may be metabolized in crude preparations, or absorbed on protein and precipitated from solution by phosphotungstic acid. Further assay in an insoluble form will lead to inaccuracies due to settling down of suspension, non-uniformity of suspension, lack of access of substrate to enzyme and presence of natural inhibitors such as phosphate (6).

Most of these errors are unavoidable if total sulfatase activity is to be assayed primarily because the major quantity of the enzymic activity could not be obtained in solution, and further attempts at treatment resulted in increasing loss of enzyme activity. However since all the above inaccuracies will minimize sulfatase activity, and the results showed greatly enhanced level in parakeratoses, it was felt that the method was acceptable for the purpose of comparative assay of scaling lesions.

A comparison was made between assay of the acetone powder and of the butanol powder and from the graph 2 it can be seen that although the linearity of the butanol powder method is better yet the activity of the butanol powder is lower at low powder concentration suggesting that the butanol method results in loss of some enzyme activity. This further implies that in all probability some activity is lost in acetone preparations, this being another source of error minimizing sulfatase levels found.



GRAPH 2. Milligram powder in 1 ml 0.006 M nitrocatechol sulfate in 0.5 M acetate buffer pH 4.9 incubated at 37° for 1 hour.

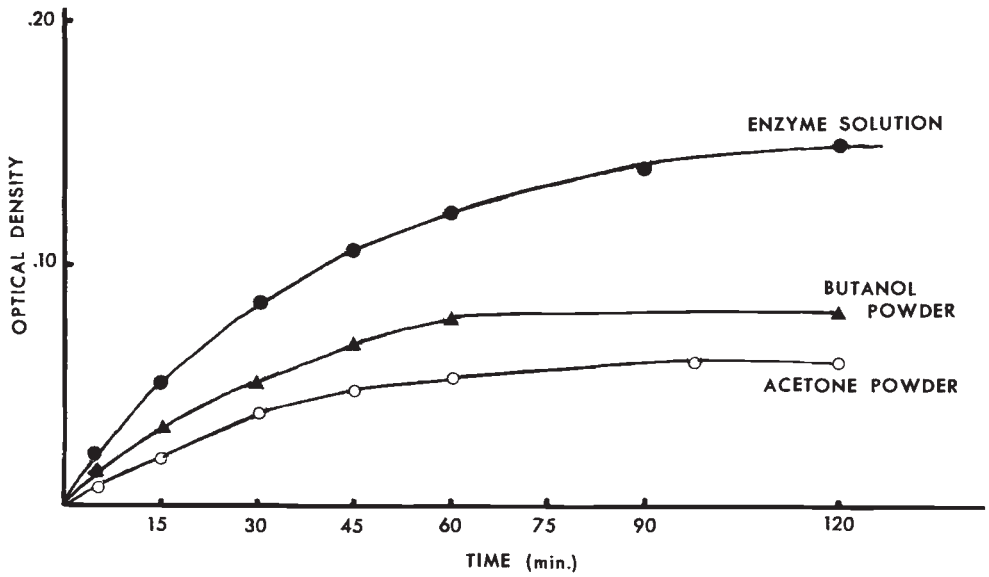


GRAPH 3. (a) 0.4 ml enzyme solution and (b) butanol powder suspension from scales and 0.6 ml 0.01 M nitrocatechol sulfate solution in 0.5 M acetate buffer—pH measured with glass electrode—and incubated for 1 hr. color developed in the usual way.

2. Solubilization

Butanol treatment or incubation with lipase followed by high speed centrifugation resulted in some of the enzyme passing into solution, the activity of the solution increasing after dialysis. Approximately 10% of the enzyme was found to be solubilized in this way, but the remainder was found to be resistant to further lipase or butanol treatment. Pepsin treatment resulted in destruction of enzyme activity. Thus there are probably at least two forms of bound enzyme, one of which is probably a lipoprotein.

In an attempt to differentiate between butanol residue and solubilized enzyme, pH-curves were studied and these showed that no distinct difference existed, the results obtained being similar to pH-curves obtained on dilution of active solutions of sulfatase A. The pH optimum found



GRAPH 4. 10 ml 0.008 nitrocatechol sulfate at pH 4.9 in 0.5 M acetate buffer and 2.0 enzyme solution or powder suspension incubated at 37°C with shaking and 1.0 ml samples pipetted into 3 ml phosphotungstic acid at given times.

TABLE 1

Inhibition expressed as percentage, control activity containing corresponding molarity of NaCl

		% Inhibition
Na F	0.1M	75
NaCN	0.1M	2
NaH_2PO_4	0.2M	70
Rat epiderm.	homogenate	nil
	acetone powder	nil
	extract powder	nil

for solution was 4.9 but there is a shift with variation in enzyme concentration again making comparative assays difficult. The pH curve corresponds to that expected for A or a mixture of A and B sulfatases and not C no peak being detected over 2 hrs in Tris buffer pH range 6.7 to 8.0 (6).

Time curves of acetone powder-soluble enzyme and insoluble residue showed that they were similar to those expected for sulfatase A solutions of relatively low activity, the activity being lower than that required to show the anomalies exhibited by this enzyme (6). They further gave no evidence of nitrocatechol metabolism suggesting that the "fall off" found with the acetone powder was due to absorption on protein.

3. Inhibition

Studies showed that fluoride was a potent inhibitor of the preparation studied whereas cyanide was not, suggesting again that sulfatase present was of the A or B type. Phosphate in insoluble preparations inhibited less than expected. This may be due to the powder form or to the presence of sulfatase C, although pH properties would be different from those obtained.

In view of reports of enzyme inhibitors present in normal epidermis (7) rat epidermis was studied for presence of inhibitors. No evidence for any sulfatase inhibitor was found (Table 1).

4. Assay Results

From tables 2 and 3 it can clearly be seen that the per mgm N sulfatase activity is greatly enhanced in both psoriasis and eczematoid parakeratoses. Furthermore, in the cases studied, there was found to be a higher level of sulfatases in psoriasis than in the other parakeratotic lesions. Rat epidermis had a higher level of sulfatase activity than human skin but still lower than that found for scales; and in this case the homogenate obtained probably resulted in a relative overestimation of rat epidermal activity.

The activity found in psoriasis is remarkably high compared to most other tissues in the

TABLE 2

1 sulfatase unit is the amount of enzyme producing 1 μg of nitrocatechol per hour under the conditions above

Disease	Activity Per GM Powder (Scales) in Sulfatase Units
Psoriasis	1000
Psoriasis	930
Psoriasis	1300
Psoriasis	1000
Psoriasis	800
Psoriasis	550
Eczema	410
Eczema	214
Eczema	490
Eczema	620

TABLE 3

Tissue	Sulfatase Units Per Mgm N
Psoriasis (scales)	5.0
Eczema (scales)	2.2
Rat epidermis	1.9
Human epidermis	0.4
Human callus	0.0

Sulfatase unit as in table 2.

human body, although very much less than that found in liver (6).

DISCUSSION

There is considerable evidence demonstrating increased esterase levels in parakeratotic horny layers notably β-glucuronidase, acid phosphatase, non specific esterases and lipases (2). It is now apparent that along with these, arylsulfatase levels are also increased.

The properties studied show that the enzyme is of the A or B type, i.e., its properties correspond to the soluble enzyme found in liver (8) rather than to the insoluble, with the major portion of the enzyme remaining insoluble. The pH optimum found suggests that the enzyme is actually A type with perhaps some B present, but fluoride inhibition if it is correct to assume that the enzyme is identical with that of the liver is suggestive of the presence of the B type. No concrete evidence for the presence of any of the C enzyme was found but to this end studies with *p*-nitrophenol sulfate should be carried out.

The function of the arylsulfatases is not known, since natural substrates have not yet been demonstrated. Still, their increase in parakeratoses (psoriasis, eczema) is almost certainly a reflection of the increased metabolism of these lesions, suggesting they may be concerned with mucopolysaccharide metabolism. In this connection it is of interest to note that it has been claimed that in psoriasis there is an increase of acid mucopolysaccharides (2, 9, 11, 12, 13), in epidermis and horny layer. It seems therefore that histochemical correlative studies staining for mucopolysaccharides and sulfatase would be of value. The difficulty of drawing any hard and fast conclusion as to whether the sulfatases then might be concerned with the synthesis or degradation of these substances rests in the disagreement as to be significant of the metachromasia, upon which staining methods for polymerized mucopolysaccharides are based.

Although the sulfatase increase seems nonspecific for parakeratoses, in the few patients studied, the level in psoriasis was significantly higher than that in other scaling lesions, and approximately 12 times higher than in normal skin.

Hence, the role played by the sulfatases in production of the parakeratotic lesion is, in the light of present knowledge, difficult to assess. It seems likely that increase in sulfatase levels is the result rather than the cause of the lesion. Although the method is very sensitive and allows working with very small quantities of material, its use in differential diagnosis is obviously limited by the similarity in levels of change in different types of parakeratoses.

SUMMARY

1. Sulfatase activity of normal and diseased epidermis was measured.
2. Sulfatase activity was found to be greatly increased in all parakeratotic lesions (scales) studied.
3. The major portion of the enzyme was found to be insoluble, with some properties corresponding to the soluble fraction of liver sulfatases.
4. The significance of increased sulfatase activity cannot yet be evaluated. Indeed, it is not unlikely that increased sulfatase levels are the result—rather than the cause—of parakeratosis.

ACKNOWLEDGMENTS

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ANNOUNCEMENT

Dr. V. J. McGovern of Sydney, Australia read his prize winning essay on "The Mechanism of Photosensitivity: An Experimental Study" at the Eightieth Annual Meeting of the American Dermatological Association, Inc. held at the Boca Raton Hotel and Club, Boca Raton, Florida. Dr. McGovern is director of the Fairfax Institute of Pathology in the Royal Prince Alfred Hospital at Sydney, Australia.

Papers for the Eleventh Annual Essay Contest should be submitted to the Secretary of the American Dermatological Association Research and Educational Fund, Inc., Dr. Wiley M. Sams, 308 Ingraham Building, Miami 32, Florida not later than November 1, 1960.